Recombinant DNA

SECOND EDITION

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EXHIBIT C

The cover illustration, by Marvin Mattelson, symbolizes some of the elements of this book. The DNA double helix is, of course, central to the book, as it is to the cover illustration. The blocks are double-stranded DNA fragments synthesized by the polymerase chain reaction, a technique that has revolutionized the way molecular genetics experiments are done. The number of fragments doubles repeatedly, going off into the distance (see Chapter 6). The coat colors of the mice running down the helix (in the same direction but with opposite polarity!), are changing from albino to chimeric, then chimeric to agouti. These coat color changes show mice in which genetic engineering has been used to knock out a specific gene. The experiment is shown more realistically in Figure 14–9.

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cloning cDNAs from an expression library (Figure 7-10). Out of a million phage plaques screened, 200 clones were identified that produced an antibody binding the antigen. Thus, with this approach, investigators were able to sample a million possible antibodies—at least a thousand times more than they could screen by conventional monoclonal antibody technology. Since phages in a particular plaque encode the antibody expressed in the plaque, it is a trivial matter to clone the heavy- and light-chain cDNAs from the phage DNA. These cDNAs can be placed into bacterial or mammalian expression vectors for production of large quantities of the selected antibody.

A recent modification of this method uses filamentous phages such as M13 instead of λ phage and allows display of the antibodies on the phage surface. This offers the advantage of being able to screen thousands more phage (because the screening can be done in solution) and to select phage that express tight-binding antibodies. We will discuss this method later and in Figure 23-10.

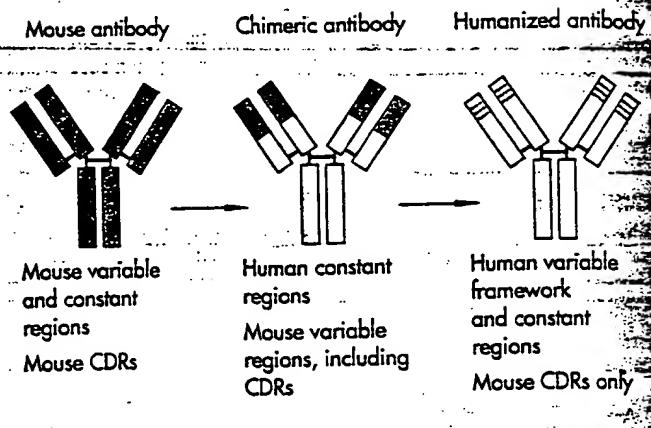

FIGURE 23-8
Antibody engineering. The basic structure of a mouse monoclonal antibody (MAb) resembles that of a human antibody. However, there are numerous differences between amino acid sequences of the antibodies from the two species. These sequence differences account for the immunogenicity of mouse MAbs in humans. A *chimeric* MAb is constructed by ligating the cDNA fragment encoding the mouse V_L and V_H domains to fragments encoding the C domains from a human antibody. Because the C domains do not contribute to antigen binding, the chimeric antibody will retain the same antigen specificity as the original mouse MAb but will be closer to human antibodies in sequence. Chimeric MAbs still contains some mouse sequences, however, and may still be immunogenic. A *humanized* MAb contains only those mouse amino acids necessary to recognize the antigen. This product is constructed by building into a human antibody the amino acids from the mouse complementarity determining regions or CDRs.

"Humanized" Monoclonal Antibodies Retain Activity But Lose Immunogenicity

Although swift progress is being made in the identification of monoclonal antibodies with potential therapeutic value, their use is limited by a problem we have already discussed in this chapter. Monoclonal antibodies are usually mouse proteins, and they are not identical to human antibodies. Thus, antibodies injected into a patient will eventually be recognized as foreign proteins and will be cleared from the circulation.

As we learned in Chapter 16, both chains of the antibody molecule can be divided into variable and constant regions. The variable regions differ in sequence from one antibody to another, and this is the region of the protein that binds the antigen. The constant region is the same among all antibodies of the same type. The first method used to reduce the immunogenicity of a mouse monoclonal antibody was simply to construct *chimeric* genes that encoded proteins in which the variable regions from the mouse antibody were fused to the constant regions from a human antibody. The chimeric antibody (Figure 23-8) retained its binding specificity but more closely resembled a natural human antibody.

This antibody, however, was not fully *humanized*, because it retained amino acid sequences from the mouse protein. Thus, scientists have set out to engineer fully humanized monoclonal antibodies that will be indistinguishable from natural molecules. Extensive studies of the three-dimensional structures of antibody molecules tell us that only a few of the one hundred amino acids in the variable region of an antibody actually contact the antigen; these regions of contact are referred to as *complementarity determining regions* (CDRs). Three CDRs each comprise the antigen-binding sites on the light and heavy chains. The rest

of the variable region serves as a scaffold to anchor the CDRs in the correct positions. This breakdown of amino acids in the variable region into those serving recognition and those serving structural roles is also evident from simply comparing the sequences of many antibody molecules. Amino acid sequences in the CDRs are *hypervariable*, whereas the structural, or *framework*, amino acids differ little.

Thus, to make a fully humanized antibody, all that would be required in principle would be to use in vitro mutagenesis to transfer the CDR amino acid sequences from a mouse MAb to a natural human antibody (Figure 23-8). This method was used to humanize an antibody that recognizes an antigen on the surface of human lymphocytes. This humanized MAb is now in clinical trials as an immunosuppressant and for treatment of lymphoid tumors. Another potentially valuable MAb binds a growth factor receptor found in large numbers on the surface of many breast tumor cells. Laboratory experiments showed that this antibody could block the growth of these cells in culture and caused tumors seeded in mice to regress. Unfortunately, the first humanized versions of this antibody bound the receptor protein but failed to block the growth of breast carcinoma cells. Investigators suspected that the problem was with the framework amino acids, and they used computer modeling to design amino acid substitutions that would strengthen the antibody-antigen interaction. Several such variant antibodies were produced and tested; one bound the receptor 250 times more tightly than did the original antibody and successfully blocked tumor cell growth in culture. This antibody is now being produced in large quantities for clinical trials.

Protein Engineering Can Tailor Antibodies for Specific Applications

Humanizing monoclonal antibodies is an example of the emerging technology of *protein engineering*, that is, a process using recombinant DNA to modify the structure of natural proteins to improve or change their function. Antibodies are particularly attractive candidates for protein engineering, because their structure is understood in great detail and because their potential for use in medicine is enormous. Another way in which antibodies are being engineered is by changing their *effector domains*, the regions of the heavy chain that specify antibody function—for example, killing of cells marked by the antibody. In this way, the mode of action of a monoclonal antibody can be reprogrammed. One promising strategy is to replace the effector domain entirely with a sequence encoding a toxin. An antibody-toxin fusion protein would deliver the toxin specifically to cells bearing the target antigen. This product could be an exceptionally potent treatment for cancer and for viral diseases such as AIDS. Antibody engineering is also being used to construct *bispecific antibodies*. In these antibodies, each of the two arms recognizes a different antigen, thus allowing an antibody to bridge the two antigens. For example, a bispecific antibody could recognize a tumor cell protein with one arm and a protein on the surface of a killer T cell with the other, thereby bringing the killer cells directly to the tumor (Figure 23-9).

Protein Engineering Is Used to Improve a Detergent Enzyme

Subtilisin is a serine protease produced by bacteria. Due to its broad specificity for proteins that commonly soil clothing, this enzyme was developed for commercial use in laundry detergents. (It is subtilisin that is prominently advertised as the enzyme additive in modern detergents.) But the first detergents containing subtilisin suffered from a serious drawback: they could not be used with bleach, because bleach inactivates the enzyme. Biochemical analysis determined that loss of activity was due to the oxidation of a methionine at position 222. Once this happened, the modified enzyme lost 90 percent of its activity. Because they knew which amino acid was bleach sensitive, however, scientists decided to see whether a variant of subtilisin could be produced that was no longer sensitive to bleach.

To do this, site-directed mutants were constructed in the gene encoding subtilisin. The strategy was simply to substitute, one at a time, each of the non–wild-type amino acids at residue 222. The mutant genes